



Published in final edited form as:

Int Rev Cell Mol Biol. 2012 ; 294: 223–258. doi:10.1016/B978-0-12-394305-7.00005-7.

CELLULAR AND MOLECULAR BIOLOGY OF OPTINEURIN

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Abstract

Optineurin is a gene linked to glaucoma, amyotrophic lateral sclerosis, other neurodegenerative diseases, and Paget's disease of bone. This review describes the characteristics of optineurin and summarizes the cellular and molecular biology investigations conducted so far on optineurin. Data from a number of laboratories indicate that optineurin is a cytosolic protein containing 577 amino acid residues. Interacting with proteins such as myosin VI, Rab8, huntingtin, transferrin receptor, and TANK-binding kinase 1, optineurin is involved in basic cellular functions including protein trafficking, maintenance of the Golgi apparatus, as well as NF- κ B pathway, antiviral, and antibacteria signaling. Mutation or alteration of homeostasis of optineurin (such as overexpression or knockdown) results in adverse consequences in the cells, leading to the development of neurodegenerative diseases including glaucoma.

Keywords

Optineurin; Glaucoma; Amyotrophic lateral sclerosis; Mutations; Molecular structure; Localization; Functional consequence

1. Introduction

Optineurin, a 67-kDa protein, has attracted much attention in the scientific world in recent years. It was first isolated in 1998 by Li et al. in yeast two-hybrid screening using an adenovirus protein E3-14.7K (group C early transcription region 3 14.7-kDa protein) as a bait and was initially named as FIP-2 (14.7K-interacting protein 2). Its expression can be induced by tumor necrosis factor- α (TNF- α) in human 293 cells and adenocarcinoma MCF-7 cells. E3-14.7K protein has been shown to inhibit TNF- α -induced apoptosis and FIP-2 can reverse the E3-14.7K protective effect in human 293 cells (Li et al., 1998). Since optineurin has a strong homology to NF- κ B essential molecule (NEMO), it was also named as NRP (NEMO-related protein) by Schwamborn et al. (2000).

The interest toward optineurin was appreciably elevated following the findings by Rezaie et al. (2002) that optineurin gene is associated with normal tension glaucoma (NTG), a subtype of primary open-angle glaucoma (POAG), one of the leading causes of irreversible bilateral blindness worldwide. It was named then as optineurin which stands for "optic neuropathy inducing" protein. More recently, mutations in optineurin are also found to be associated with amyotrophic lateral sclerosis (ALS; Maruyama et al., 2010). Optineurin is further noted to be localized in pathological structures in ALS, neurofibrillary tangles and dystrophic neuritis in Alzheimer's disease, Lewy bodies and Lewy neuritis in Parkinson's disease, ballooned neurons in Creutzfeldt-Jakob disease, glial cytoplasmic inclusions in multiple system atrophy, and Pick bodies in Pick disease (Osawa et al., 2011). In addition, optineurin is identified as one of the genetic risk factor for Paget's disease of bone (PDB) (Albagha et al., 2010; Chung et al., 2010).

This review describes the characteristics of optineurin protein and summarizes the cellular and molecular biology work performed on optineurin in recent years. Data from a number of laboratories indicate that optineurin is involved in basic cellular functions such as protein trafficking, maintenance of the Golgi apparatus, as well as NF- κ B pathway and antiviral signaling. Mutation or alteration of homeostasis of optineurin (such as overexpression or knockdown) results in adverse consequences in the cells, leading to the development of neurodegenerative diseases including glaucoma.

2. Molecular Structure and Protein Characteristics

2.1. Genomic DNA

The human optineurin gene is located at chromosome 10:13142082-13180276 (gene ID: 10133) and spans about 37kb genomic region. Four different transcripts (NM_001008211.1, NM_001008212.1, NM_001008213.1, and NM_001008214.1) have been reported, each containing a different 5'-untranslated region (UTR), but all have the same open reading frame.

The detailed optineurin promoter structure is unknown. Sudhakar et al. (2009) cloned about 1kb of DNA fragment upstream of the human optineurin cDNA sequence. This putative promoter includes 221bp of exon 1. Analysis of this promoter showed several putative Sp1 sites and one NF- κ B site located immediately upstream of the transcription start site. No TATA box or initiator element is present in this promoter but putative binding sites for heat shock factors, HSF1 and HSF2, MyoD, neuron-restrictive silencing factor (NRSF, also known as REST), and cyclic AMP response element-binding protein (CREB) were identified (Sudhakar et al., 2009). This putative promoter was activated upon treatment of HeLa and A549 cells with TNF- α . A smaller promoter from -136 to +221 was in addition made which contained the putative Sp1 and NF- κ B sites. Similar to the 1kb full-length promoter, the smaller 0.36kb fragment can also be activated by TNF- α in A549 and HeLa cells. Interestingly, the smaller fragment showed more basal activity than the full-length 1kb promoter, suggesting that a negative regulatory element is present in sequences upstream of the minimal promoter.

2.2. mRNA and gene expression during embryogenesis

The human optineurin gene contains 3 noncoding exons in the 5'-UTR region and 13 exons that code for a 577-amino acid protein. Alternative splicing at the 5'-UTR generates at least four different isoforms, but all have the same open reading frame (Rezaie and Sarfarazi, 2005). The synonyms that have been used to describe this gene include: FIP-2, GLC1E, NRP, HIP7, HYPL, and TFIIIA-INTP.

Optineurin transcripts are ubiquitously present in various tissues in the adult mouse. It is expressed during early stages of eye development in mouse. At both 10.5 and 13.5 days postconception, a strong specific expression was detected by *in situ* hybridization in the optic vesicle (De Marco et al., 2006; Rezaie et al., 2007). During embryogenesis, optineurin may play a pivotal role in the overall development of the eye.

2.3. Protein

2.3.1. Domains—Figure 5.1 shows sequence alignment of optineurin protein from human, chimpanzee, cow, dog, rat, mouse, chicken, and zebrafish. Figure 5.2 presents phylogenetic tree to depict the distance between different species. A high amino acid homology exists in optineurin from different species (Rezaie and Sarfarazi, 2005; Rezaie et al., 2005). This protein contains several putative domains including a NEMO-like domain, at least one leucine zipper, multiple coiled-coil motifs, an ubiquitin-binding domain (UBD), a

microtubule associated protein 1 light chain 3 (LC3)-interacting motif (LIR) (Wild et al., 2011), and a carboxyl (C)-terminal C₂H₂ type of zinc finger (Hattula and Peranen, 2000; Li et al., 1998; Schwamborn et al., 2000; Stroissnigg et al., 2002; Fig. 5.3A).

Optineurin was reported in earlier studies (Rezaie et al., 2002; Sarfarazi and Rezaie, 2003) to be present in the aqueous humor and culture medium and was suspected to be a secreted protein. However, Kroeber et al. (2006) did not detect any optineurin in the aqueous humor in their transgenic mice overexpressing wild-type optineurin under the control of lens specific β B1-crystalline promoter (β B1-crystallin-OPTN mice). Subsequent secretion assays also showed that optineurin is not secreted in human trabecular meshwork (TM) and retinal pigment epithelial (RPE) cultures, even under conditions in which secretion of proteins such as myocilin is promoted or when optineurin is overexpressed (Park et al., 2006). Optineurin is thus concluded not to be a secreted protein. The difference in results could be related to specificity of the antibody used.

2.3.2. Posttranslational modification—Optineurin is neither *N*- nor *O*-glycosylated (Ying et al., 2010). When subjected to membrane protein extraction, optineurin in total lysates of RGC5 cells (a transformed cell line used as a model for retinal ganglion cells or RGCs) distributed exclusively in the hydrophilic fraction. Optineurin is therefore not a membrane protein but rather a cytosolic protein, consistent with the prediction that the protein sequence contains no obvious transmembrane domains (Ying et al., 2010). Optineurin possesses an UBD, is ubiquitinated, and is processed through the ubiquitin-proteasome pathway (Shen et al., 2011; Ying et al., 2010).

The endogenous optineurin is phosphorylated (Ying et al., 2010). Using stable isotope labeling with amino acids in cell culture (SILAC)-based mass spectrometry, the endogenous optineurin was found to be phosphorylated at Ser¹⁷⁷ which is adjacent to the optineurin LC3 interacting LIR site (Wild et al., 2011). Multiple phosphorylated LIR peptides with up to three phosphorylated groups were also identified in the presence of overexpressed TANK-binding kinase 1 (TBK1) in mouse embryo fibroblasts (Wild et al., 2011).

2.3.3. Oligomerization—By native blue gel electrophoresis, optineurin is shown to be capable of forming 420kDa homo-oligomers, which, based on the 67kDa monomer size, is estimated to be hexamers (Ying et al., 2010). In addition, optineurin interacts and associates with Rab8, myosin VI, and transferrin receptor (Chibalina et al., 2008; Ying et al., 2010), either in singly or in combination, to form supermolecular complexes with sizes larger than 400kDa.

2.3.4. Cellular localization—Optineurin is ubiquitously expressed in many tissues including the heart, brain, placenta, skeletal muscle, kidney, pancreas, adrenal cortex, liver, and the eye (Li et al., 1998; Rezaie and Sarfarazi, 2005; Rezaie et al., 2005). In the eye, optineurin is expressed in the TM, nonpigmented ciliary epithelium, and remarkably in retina (Rezaie and Sarfarazi, 2005). A study (Kroeber et al., 2006) using transgenic mice indicated that the retina staining is confined to neurons and that RGC is labeled with a high intensity. The strong RGC labeling was confirmed in another investigation (De Marco et al., 2006).

The endogenous optineurin generally is localized in the cytoplasm with a diffuse distribution pattern. Of note is that the immunostaining patterns may depend on the antibodies and washing procedures used. There are two commercially available antibodies, anti-C-terminal- and anti-INT-optineurin. The former is reactive to human, mouse, and rat optineurin, while the latter is reactive only to the human protein. In both RGC5 and human APRE-19 RPE cells, a diffuse, cytoplasmic distribution pattern of optineurin resulted when they were

washed in glycine-containing solution and stained with anti-C-terminal-optineurin antibody. When glycine was omitted in the rinse, the cytoplasmic staining was low and optineurin appeared to locate largely in the perinuclear area overlapping with the Golgi marker GM130. Anti-INT-human optineurin antibody, presumably reactive against epitope(s) different from those of anti-C-terminal antibody, yielded the diffuse, cytosolic pattern regardless whether glycine was used for the rinse in human RPE cells. It was concluded that while optineurin has a diffuse, cytoplasmic distribution pattern, a population of the protein is associated with the Golgi apparatus. As optineurin is shown not to be a membrane protein, the optineurin–Golgi association is probably indirect via interactions of other Golgi-associated proteins such as huntingtin (Htt) and Rab8 (Ying et al., 2010).

When overexpressed after transfection, forced expressed exogenous optineurin accumulates, appearing as dots or granular structures, termed foci, around the Golgi complex. The foci, seemingly residing in close proximity, essentially do not colocalize with the Golgi complex (Park et al., 2006; Ying et al., 2010).

2.3.5. Cellular processing—By pulse chase experiments, the endogenous optineurin was found to be a relatively short-lived protein with a half life of about 8h (Ying et al., 2010) in RGC5 cells. The endogenous optineurin is ubiquitinated and its level in neuronal RGC5 and PC12 cells is increased by treatment of proteasomal inhibitors but not by autophagic and lysosomal inhibitors, indicating that the ubiquitin–proteasome system is the major pathway for endogenous optineurin processing. Autophagy and lysosomes have a rather minor role (Shen et al., 2011).

3. Optineurin-Binding Partners

Optineurin has been shown to interact with a number of proteins (Fig. 5.3A) including Rab8, huntingtin, myosin VI (Hattula and Peranen, 2000; Sahlender et al., 2005), transferrin receptor (Park et al., 2010), metabotropic glutamate receptor (Anborgh et al., 2005), transcription factor IIIA (TFIIIA) (Moreland et al., 2000), serine/threonine kinase receptor-interacting protein 1 (RIP1) (Zhu et al., 2007), and TBK1 (Morton et al., 2008) implicating roles of optineurin in multiple cellular functions.

3.1. Rab8

Rab proteins belong to a large family of small guanosine triphosphatases (GTPases) that participate and regulate various membrane transport pathways. Each Rab protein has a distinct location corresponding to the pathway it regulates (Stenmark and Olkkonen, 2001; Zerial and McBride, 2001). Rab8, a binding partner of optineurin, has been shown to regulate polarized membrane trafficking pathways from the Golgi complex to the cell surface (Huber et al., 1993a,b; Moritz et al., 2001) and promote changes in cell shape by reorganizing actin and microtubules (Huber et al., 1993b; Peranen et al., 1996). The constitutively active GTP-bound mutant form of Rab8-Q67L, but not the dominant-negative GDP-bound mutant form of Rab8-T22N, interacts with the amino (N)-terminal region (amino acids 141–209) of optineurin (Hattula and Peranen, 2000). This region of optineurin contains the leucine zipper domain. Optineurin and Rab8 are suggested to form a complex that participates in regulation of the post-Golgi transport of proteins, the sorting of which is mediated by the clathrin adaptor complex 1 (Au et al., 2007; Sahlender et al., 2005).

3.2. Huntingtin

Htt is a huge cytosolic protein (3144 amino acids) associated with Huntington's disease. The abnormal polyglutamine expansion in the N-terminal region of Htt produces significant dysfunction and neural death, especially in the medium spiny neurons of the striatum

(Kremer et al., 1994). Subcellular fractionation and microscopic studies have shown that Htt is associated with vesicles and microtubules (DiFiglia et al., 1995; Hoffner et al., 2002) by interacting with HAP1, Htt-associated protein 1, which is reported to form a complex with the dynactin subunit p150^{glued} and modulate or regulate the dynein–dynactin complex. There is evidence that Htt participates in post-Golgi trafficking of proteins that follow the regulated secretory pathway (del Toro et al., 2006). Deletion experiments by Hattula and Peranen (2000) showed that Htt directly binds to a C-terminal (amino acids 411–461) region of optineurin. Optineurin colocalizes with Htt in the Golgi apparatus, linking Htt to Rab8 (Sahlender et al., 2005). Coexpression of optineurin and Htt enhanced the recruitment of Htt to Rab8-positive vesicular structures. Post-Golgi trafficking to lysosomes is impaired in cells expressing mutant Htt by delocalizing optineurin/Rab8 complex from the Golgi apparatus (del Toro et al., 2009).

3.3. Myosin VI

Myosin VI is a multifunctional actin-based motor protein found in a number of intracellular compartments including endocytic vesicles (Aschenbrenner et al., 2003; Buss et al., 2001), membrane ruffles (Buss et al., 1998), the Golgi complex, and secretory vesicles (Buss et al., 1998). It plays a role in the basolateral delivery of membrane proteins (Au et al., 2007; Buss et al., 2001, 2004; Spudich et al., 2007; Warner et al., 2003). Using the tail of myosin VI as a bait in a yeast two-hybrid screening of human umbilical vein epithelial cell cDNA library, a 375-amino acid C-terminal fragment of optineurin was identified as a myosin VI-binding partner (Sahlender et al., 2005). Further deletion studies indicated that the myosin VI-binding site is between amino acids 412 and 520 which interacts with the Arg-Arg-Leu (RRL) sequence in the tail domain of myosin VI. Knockdown optineurin by siRNA caused a marked reduction in the amount of myosin VI associated with the Golgi complex, fragmentation of the Golgi, and a reduction in exocytosis of vesicular stomatitis virus G-protein to the plasma membrane. Optineurin was thus suggested to mediate the targeting of myosin VI to the Golgi complex and play a role in organization of the Golgi apparatus and exocytosis (Sahlender et al., 2005). Subsequent studies have established that both myosin VI and optineurin are required in the final stages of the secretory pathway for the fusion of secretory vesicles with the plasma membrane (Bond et al., 2011).

3.4. Transferrin receptor

Immunoprecipitation experiment established that transferrin receptor interacts with optineurin (Park et al., 2010). The binding domain is yet to be specified. It has been shown that sequestration of transferrin receptor upon overexpression of optineurin may be a factor that leads to impairment of the transferrin uptake in cells (Park et al., 2010).

3.5. Metabotropic glutamate receptors 1 and 5 (mGluR1 and mGluR5)

mGluRs that are linked to the activation of phospholipase C (PLC), increase in intracellular inositol 1,4,5-triphosphate (IP₃) formation, and the release of Ca²⁺ stores inside the cells play an important role in regulating neuronal function (Conn and Pin, 1997). They belong to a subfamily of G-protein-coupled receptors and are divided into three groups based on the homology of their amino acid sequences. mGluR1 and mGluR5 are group I members and each has several splice variants [mGluR1 has four: a (or α), b (or β), c, and d. mGluR5 has two: a and b]. Optineurin has been shown to be a group I mGluR-interacting protein (both mGluR1 and mGluR5) (Anborgh et al., 2005) that functions to inhibit mGluR-G-protein coupling to PLC and IP₃ signaling (Anborgh et al., 2005). Htt coprecipitates with mGluR1 (probably through optineurin) and when wild type (Q15) and mutant (Q135) Htt were coexpressed with optineurin, only the latter resulted in augmented optineurin binding to mGluR1a and increased optineurin-mediated attenuation of mGluR1a signaling (Anborgh et al., 2005).

3.6. Transcription factor IIIA (TFIIIA)

TFIIIA activates 5S ribosomal RNA gene transcription in eukaryotes. It is a single protein that contains zinc and possesses repetitive C₂H₂ zinc finger domains (Clemens et al., 1993; Theunissen et al., 1992). Optineurin protein was identified in the yeast two-hybrid system using the C-terminal portion of *Xenopus* TFIIIA as a bait (Moreland et al., 2000). The interaction was verified by co-immunoprecipitation *in vitro* and by cochromatography (Moreland et al., 2000). The TFIIIA-interacting region is likely within the central leucine-rich domain on optineurin. The complex of the two proteins (as evidenced by cochromatography) is stable at high salt inferring a hydrophobic nature for the TFIIIA–optineurin interaction (Moreland et al., 2000). The possible role optineurin in the 5S ribosomal RNA gene transcription remains to be determined.

3.7. Serine/threonine kinase receptor-interacting protein 1 (RIP1)

NF- κ B is sequestered in the cytoplasm in a complex with inhibitory I κ B protein. Upon stimulation by cytokines such as TNF- α , signaling intermediates TRADD (TNF receptor-associated death domain), TRAF2 (TNF receptor-associated factor 2), and RIP1 are recruited to the TNF receptor (TNFR), resulting in the assembly of a signaling complex. In this complex, RIP1 is rapidly ubiquitinated with Lys(K)63-linked polyubiquitin (polyUb) chains and binds with the UBD domain of the regulatory subunit of I κ B kinase (IKK) complex, IKK γ (also known as NEMO). This leads to activation of two catalytic subunits, IKK α and IKK β , of IKK to trigger the phosphorylation, ubiquitination, and degradation of I κ B. NF- κ B is then translocated into nucleus to induce transcription of target genes (Ea et al., 2006; Wu et al., 2006). NEMO is thus an essential component for NF- κ B signaling. Optineurin has a high homology with NEMO (53%). It has a K63-linked polyUb-binding domain similar to that of NEMO. A point mutation Asp⁴⁷⁴ \rightarrow Asn (D474N) (D311N in NEMO) in the UBD domain of optineurin abolishes its binding to K63-linked polyUb. Optineurin has been shown to compete with NEMO to bind with ubiquitinated RIP which is necessary for the efficient activation of NF- κ B induced by TNF- α (Zhu et al., 2007). Overexpression of optineurin competitively inhibited TNF- α -induced activation of NF- κ B. On the other hand, microRNA silencing of optineurin resulted in markedly enhanced TNF- α -induced NF- κ B activation. This indicates that optineurin is a negative regulator of TNF- α -induced NF- κ B activation (Zhu et al., 2007) via binding with polyUb RIP.

3.8. TAX1, TAX2, and TAX1BP1

TAX1 is one of the nonstructural genes human T-cell leukemia virus type 1 (HTLV-1) encoded. TAX2 is the equivalent of TAX1 in HTLV-2 and TAX1 and TAX2 have many shared activities. Both coprecipitate with optineurin and TAX1 has the same subcellular distribution as optineurin (Journo et al., 2009). The UBD domain of optineurin is required for its binding to TAX1. A point mutation D474N in the UBD domain of optineurin severely reduces the interaction of optineurin with TAX1 (Journo et al., 2009).

Ubiquitination of TAX1 is required for its interaction with optineurin. Binding with optineurin can stabilize TAX1 polyubiquitination and ubiquitination-defective TAX1 mutants exhibit impaired binding to optineurin. TAX1 has been known to bind NEMO, triggering the activation of IKK α and IKK β (Chu et al., 1999; Harhaj and Sun, 1999; Jin et al., 1999) and in turn activation of NF- κ B. Since optineurin is NEMO-like, it is not surprising that optineurin is also capable of potentiating the activation of NF- κ B by TAX1.

Tax1-binding protein 1 (TAX1BP1) is a binding partner of TAX1 reported to interact with A20, Itch and ring finger protein 11 (RNF11) to form a functional ubiquitin-editing complex that regulates the ubiquitination of RIP1 and TRAF6 (Shembade et al., 2007). Interestingly, TAX1BP1 itself also interacts with optineurin. When TAX1BP1 is present, the interaction

between optineurin and TAX1 is strongly induced and the interaction between optineurin and TAX1BP1 is also increased by coexpression of TAX1, suggesting that these three proteins interact with each other to form a ternary complex (Journo et al., 2009). RNF11 interacts with optineurin (Azmi and Seth, 2005) likewise. It is unclear though whether RNF11 is also present in the TAX1BP1/TAX1/optineurin complex and whether it regulates the TAX1 ubiquitination.

3.9. CYLD

CYLD was originally identified as a tumor suppressor gene mutated in familial cylindromas (turban tumor syndrome). This gene encodes a cytoplasmic protein that functions as a deubiquitinating enzyme. It is a NEMO-interacting protein and thus a negative regulator of NF- κ B activation (Kovalenko et al., 2003; Trompouki et al., 2003). It has been demonstrated that via its deubiquitinase activity, CYLD specifically catalyses cleavage of K63-linked polyUb chains from its target proteins such as RIP, NEMO, and TRAFs to prevent NF- κ B activation (Brummelkamp et al., 2003; Nagabhushana et al., 2011). Using full-length optineurin as a bait, CYLD was identified as optineurin-interacting protein by yeast two-hybrid screening (Nagabhushana et al., 2011). Deletion analysis showed that the C-terminal region of optineurin that contains an UBD domain (424–509 amino acids) is involved in binding to CYLD (Fig. 5.3A). A point mutation His⁴⁶⁸ \rightarrow Arg (H486R) in the UBD domain of optineurin abolishes its interaction with CYLD. Deletions of various domains of CYLD revealed that amino acids 460–592 of CYLD which encompass a cytoskeleton associated protein glycine-rich (CAP) domain are sufficient for interaction with optineurin (Nagabhushana et al., 2011). Mediating the interaction between CYLD and polyUb RIP, optineurin may act as an adaptor protein bringing CYLD and the CYLD substrate RIP together to facilitate deubiquitination of ubiquitinated RIP by CYLD (Nagabhushana et al., 2011).

3.10. TANK (TRAF-associated NF- κ B activator) binding kinase 1 (TBK1)

TBK1, TANK-binding kinase 1 or TNF- α -activated protein kinase 1, is a member of the IKK subfamily of protein kinase. TBK1 becomes activated in response to lipopolysaccharide or viral double-stranded DNA. It phosphorylates the transcription factor interferon regulatory factor 3 (IRF3), induces its translocation to the nucleus, and stimulates transcription of the genes coding type-1 interferons (Hemmi et al., 2004; Matsui et al., 2006; McWhirter et al., 2004). TBK1 can also be recruited to the TNF receptor complex in response to TNF- α (Kuai et al., 2004) and activate NF- κ B-directed transcription (Bonnard et al., 2000).

TBK1 was identified as an optineurin binding partner by yeast two-hybrid screens using optineurin as a bait (Morton et al., 2008). The TBK1 binding site is located between residues 1 and 127 of optineurin and the optineurin-binding domain is localized to C-terminal domain residues 601–729 of TBK1 (Morton et al., 2008). Protein sequence databases search disclosed that the amino acid sequence between residues 78 and 121 of optineurin is homologous to the TBK1-interaction domain present in three other binding partners for TBK1, TANK, NAP1 (IKK-related kinase- or NAK-associated protein 1), and SINTBAD (similar to NAP1, TBK1 adaptor). It is likely that optineurin, TANK, NAP1, and SINTBAD all bind to the same region of TBK1 at the common TBK1-binding domain.

Optineurin has been demonstrated to have a role in the inhibition of virus-triggered interferon- β induction (Mankouri et al., 2010). TBK1–optineurin interaction may be involved in the antiviral signaling pathways.

3.11. LC3/GABARAP

Microtubule associated protein 1 light chain 3 (LC3) is an autophagy protein (mammalian homolog of yeast Atg8) required in the formation of autophagosomes (Kabeya et al., 2000). Autophagy is initiated by the formation of a cup-shaped membrane (termed phagophore or isolation membrane) that has been recently noted to originate from the endoplasmic reticulum (Hayashi-Nishino et al., 2009; Yla-Anttila et al., 2009). The phagophore then envelops parts of the cytoplasm to form a double-membrane vesicle (termed autophagosome), which eventually fuses with the lysosomes/vacuole to form autolysosomes (Weidberg et al., 2010). LC3 conjugates with phosphatidylethanolamine to form lipidated LC3 (LC3-II) before association with the membrane. It then localizes to the nascent and early autophagic vacuole membranes. After autophagosomes seal, the LC3 attached to the outer membrane is cleaved off and the LC3 on the inner membrane is trapped inside the sealed autophagosomes (Eskelinen, 2005). γ -Aminobutyric acid receptor-associated protein (GABARAP) is also an Atg8 homologue in the mammalian system. Similar to LC3, GABARAP subjects to a C-terminal modification process and is involved in autophagosomal membrane formation (Chakrama et al., 2010; Kabeya et al., 2004).

LC3/GABARAP was recently identified to be an optineurin binding partner (Wild et al., 2011). The specific interactions between optineurin and LC3/GABARAP proteins were verified by pull down assays in mammalian cells, directed yeast two-hybrid transformations, and *in vitro* pull down using purified proteins (Wild et al., 2011). Deletion experiment on optineurin pinpointed an LC3 interacting LIR motif which is located between the coiled-coil domains of optineurin encompassing amino acids 169–209. This LIR motif contains a linear tetrapeptide sequence which is also present in known autophagy receptors (such as NBR1, P62/SQSTM1, NIX/BNIP3L, and CALR) (Wild et al., 2011).

Optineurin is localized in LC3-positive vesicles upon induction of autophagy. A single point mutation of Phe¹⁷⁸ → Ala (F178A) or Ile¹⁸¹ → Ala (I181A) abrogated the colocalization and the interaction between optineurin and LC3/GABARAP (Wild et al., 2011). A similar abrogation was also observed with ubiquitin binding-deficient optineurin variants. Optineurin is thus concluded to serve as an autophagy receptor that binds with LC3/GABARAP via a phenylalanine-containing LIR motif and ubiquitin via its UBD domain, allowing optineurin to be involved in selective autophagy of ubiquitin-coated cytosolic *Salmonella enterica*. TBK1 is shown to phosphorylate optineurin on Ser¹⁷⁷, enhancing thereby the LC3 binding by optineurin and promoting autophagic clearance of *Salmonella*. Silencing optineurin or TBK1, or LC3- or ubiquitin-binding-deficient optineurin mutants impaired *Salmonella* autophagic clearance, resulting in increased bacterial proliferation (Wild et al., 2011).

3.12. Others

Other optineurin binding proteins may include: A20, ABIN1, TRAF3 (Mankouri et al., 2010), TBC1D17, UXT, ZBTB33, and BAT4. Further, investigations will be needed to elucidate the physiological and pathological significance of these interactions.

4. Optineurin Mutations Associated with Diseases (Fig. 5.3B)

4.1. Glaucoma

Glaucoma is a major cause of blindness characterized by degeneration of the optic nerve, RGC death (Kerrigan et al., 1997; Kuehn et al., 2005), and progressive axonal and visual field loss (Quigley, 2011). It is estimated that by the year of 2020, the number of affected population will rise to around 79.6 million (Quigley and Broman, 2006). It is a slow disease, usually bilateral but unequal in degree. The most common form of glaucoma, adult-onset

POAG, is typically associated with an elevation of the intraocular pressure (IOP) and characteristic optic nerve degeneration. A subset of POAG patients, however, may manifest optic nerve damage even without an IOP increase, and this form of open-angle glaucoma is called normal tension glaucoma or NTG (Anderson, 2003; Hoyng and Kitazawa, 2002).

POAG is genetically heterogeneous caused by several susceptibility genes and perhaps also environmental factors (Allingham et al., 2009; Kwon et al., 2009; Wang et al., 2001; Wiggs, 2007). Currently, a total of 15 chromosomal loci, designated as GLC1A to GLC1O, have been linked to POAG. Four candidate genes identified so far include myocilin (GLC1A) (Stone et al., 1997), WD40-repeat36 (GLC1G) (Rao et al., 2011), optineurin (GLC1E) (Rezaie et al., 2002), and neurotrophin-4 (NTF-4) (GLC1O) (Rao et al., 2011).

Rezaie and coworkers in 2002 studied 54 families with autosomal dominantly inherited adult-onset POAG (the majority of these families presented with normal IOP) and identified the causative gene on chromosome 10p14 and designated it optineurin (OPTN). This gene was previously identified as FIP-2 (Li et al., 1998) and NRP (Schwamborn et al., 2000). DNA sequence analyses detected four mutations in patients with POAG: Glu⁵⁰ → Lys (E50K), Met⁹⁸ → Lys (M98K), Arg⁵⁴⁵ → Gln (R545Q), and 691_692insAG (2-bp “AG” insertion). These mutations were reported to be responsible for 16.7% of hereditary forms of NTG with an additional attributable risk factor of 13.6% in both familial and sporadic cases (Rezaie et al., 2002).

Subsequent studies indicated that optineurin sequence alterations do not have a strong correlation with high tension POAG (Alward et al., 2003; Ayala-Lugo et al., 2007; Wiggs et al., 2003) and that the frequency of coding and intronic polymorphisms within the optineurin gene in NTG populations varies with ethnic background (Baird et al., 2004; Caixeta-Umbelino et al., 2009; Hauser et al., 2006; Leung et al., 2003; Liu et al., 2008; Mukhopadhyay et al., 2005; Sarfarazi and Rezaie, 2003; Tang et al., 2003; Toda et al., 2004; Weisschuh et al., 2005; Yen et al., 2008). The 691_692insAG optineurin variant is rare (Ayala-Lugo et al., 2007). The R545Q variation is likely to be a non-disease-causing polymorphism. The M98K change may be associated with a fraction of NTG in patients of Japanese ethnicity (Alward et al., 2003). E50K is associated with NTG patients in Caucasian and Hispanic populations but the prevalence is found much lower than originally reported by Rezaie et al. (2002). Overall, the E50K frequency is reported to be between 0.1% and 13.5% (Ayala-Lugo et al., 2007) in POAG cases, afflicting an estimated 67,000 to 9 million patients. E50K mutation does, however, seem to be associated with a more progressive and severe disease (Aung et al., 2005; Hauser et al., 2006). In addition to the genetic link, a causal role of E50K in glaucoma is also implicated by a recent study of Chi et al. (2010) that loss of RGCs, reduction of retinal thickness, and excavation of the optic nerve head are observed in E50K-expressing normal tension transgenic mice.

Other optineurin gene alterations observed in various patient population include: a missense mutation Lys³²² → Glu (E322K) in exon 10 of optineurin in Chinese POAG family (Xiao et al., 2009); His²⁶ → Asp (H26D) alteration (Funayama et al., 2004; Fuse et al., 2004) in Japanese POAG families; Glu¹⁰³ → Asp (E103D), Val¹⁴⁸ → Val (V148V), and IVS13+21C → G in the sporadic Chinese patients with POAG (Leung et al., 2003), and H486R in POAG (Leung et al., 2003) and juvenile open-angle glaucoma (Willoughby et al., 2004) families.

4.2. Amyotrophic lateral sclerosis

ALS is a progressive disorder characterized by degeneration of motor neurons of the primary motor cortex, brainstem, and spinal cord (Leigh, 2007). It is a genetically heterogeneous disease. Genes that encode TAR DNA-binding protein of 43kDa (TDP-43)

protein (Sreedharan et al., 2008), Cu/Zn superoxide dismutase (SOD-1) (Rosen et al., 1993), angio-genin (Greenway et al., 2006), vesicle-associated membrane protein (Nishimura et al., 2004), valosin-containing protein (Johnson et al., 2010), and fused in sarcoma/translated in liposarcoma (FUS, also known as TLS) (Kwiatkowski et al., 2009; Vance et al., 2009) are reported to be responsible for some of the classic familial ALS.

Maruyama et al. (2010) reported three mutations in the gene encoding optineurin in Japanese familial or sporadic ALS patients: a homozygous deletion of exon 5, a homozygous nonsense Gln³⁹⁸ stop (Q398X, in exon 12), and a heterozygous missense Glu⁴⁷⁸ → Gly (E478G, in exon 14) mutation. In the gene with the deletion of exon 5, if there was a transcript, the transcript splicing from exon 4 to exon 6 would cause a frame shift and make a stop codon, which would be expected to translate a peptide 58 amino acids in length. Q398X mutation generates a premature stop codon at amino acid 398, truncating the 577 amino acid optineurin protein to one of 397 amino acids in length. This truncation results in a deletion of the coiled-coil 2 domain. E478G mutation is located in the UBD domain. This glutamic acid is highly conserved among optineurin proteins of a wide range of species and is situated within the DFxxER motif of the UBD domain shared among optineurin, NEMO, and A20 binding and inhibitor of NF-κB protein. The Q398X nonsense mutation and probably the exon 5 deletion mutation as well would cause a decrease in optineurin expression resulting from nonsense-mediated mRNA decay of the transcript carrying the nonsense mutations. The mechanism of recessive mutation causing ALS is expected to be loss of function. On the other hand, the E478G missense mutation increased the immunoreactivity for optineurin in the cell body and neurites. The increased amount and different distribution of the mutated protein might disturb neuronal functions, and accelerate the inclusion body formation in sporadic ALS.

A heterozygous nonsense 382_383insAG (2-bp “AG” insertion, also called 691_692insAG) mutation and a novel missense mutation Arg⁹⁶ → Leu (R96L) were reported in French familial ALS patients (Millecamps et al., 2011). The 382_383insAG was also previously described as a dominant mutation responsible for familial POAG in Japan (Rezaie et al., 2002) and Eastern Europe (Ayala-Lugo et al., 2007). This mutation presumably induces a premature stop codon in exon 6. The level of optineurin was decreased in patients with this mutation, and a loss of optineurin function was predicted. The missense mutation R96L on the contrary might lead to a gain of function, although no accumulation of the optineurin protein was detected in the patients' lymphoblasts.

Van Blitterswijk et al. (2011) screened a large Dutch cohort of sporadic ALS patients and identified a nonsense Gln¹⁶⁵ stop (Q165X) and a missense Gln⁴⁵⁴ → Glu (Q454E) mutations. Because the Q165X mutation would probably result in a 72% truncated optineurin protein, binding to Rab8, mGluR1a, TFIIIA, Htt, and myosin VI might be eradicated. The Q454E mutation is located near the area that contains binding sites for Htt and myosin VI. The localization of these two mutations and their predicted effects suggest that they are pathogenic. Mutations, however, were not detected in the Dutch familial ALS patients, underscoring that the genetic background of ALS may differ between different populations.

Optineurin was also shown to associate with another ALS-related protein, FUS (Ito et al., 2011) and myosin VI in the basophilic inclusions of familial ALS with FUS mutation and in neurodegenerative basophilic inclusion body disease. FUS has a role in intracellular trafficking in collaboration with myosin VI and is also known to act as a coactivator of NF-κB (Uranishi et al., 2001). Dysregulation of these pathways by FUS and optineurin is conceivably also an underlying factor for pathology.

4.3. Other neurodegenerative diseases

Optineurin was shown to be located not only in the skein-like inclusions and round hyaline inclusions in ALS but also in the senile plaques and neurofibrillary tangles in Alzheimer's disease, Lewy bodies and Lewy neuritis in Parkinson's disease, ballooned neurons in Creutzfeldt–Jakob disease, glial cytoplasmic inclusions in multiple system atrophy, and Pick bodies in Pick disease. This indicates that optineurin is widely distributed in neurodegenerative conditions (Osawa et al., 2011). The significance of such a finding, however, is unknown. Optineurin aggregates are generally found to be ubiquitin positive, and the optineurin aggregation may be the common process involved in neurodegeneration and cell death. Optineurin may be itself an aggregation-prone protein present in the affected neurons and glia. On the other hand, optineurin could also be just secondarily entrapped in the inclusion bodies in various conditions or in ubiquitin.

4.4. Paget's disease of bone

PDB is one of the most frequent metabolic bone disorders (with a prevalence between 1% and 5%) affecting individuals above age 55 in Caucasian population (Altman et al., 2000; Chung et al., 2010; van Staa et al., 2002). This disease is characterized by focal areas of increased and disorganized bone remodeling that can cause bone pain, bone deformity, pathological fracture, deafness, and secondary osteoarthritis (Siris, 1998). The cause of PDB is not completely understood, although both genetic and environmental factors are implicated (Lucas et al., 2008). Between 15% and 40% of individuals with PDB have an affected first-degree relative (Morales-Piga et al., 1995). Current evidence suggests that this disease is genetically heterogeneous, resulting from mutations in several disease genes. One such gene, the ubiquitin-associated domain of *Sequestosome 1* (SQSTM1), was identified and its mutations were noted to affect about 10% of the patients with sporadic or familial PDB (Beyens et al., 2004; Falchetti et al., 2004; Hocking et al., 2002; Laurin et al., 2002). Albagha et al. (2010), using a genome-wide association approach to screen genetic variants that predispose to PDB in individuals without SQSTM1 mutations, identified CSF1 gene at the 1p13 locus, optineurin gene at 10p13, and TNFRSF11A gene at 18q21 as candidate genes for disease susceptibility (Albagha et al., 2010). Optineurin may have an unrecognized role in regulating the bone metabolism.

5. Possible Cellular Function of Optineurin and Dysfunctions Caused by Optineurin Mutations

5.1. Neuroprotection or neurotoxicity?

Optineurin, when transfected into NIH3T3 cells, has been noted to increase cell survival, protecting them from hydrogen peroxide-induced cell death and blocking cytochrome C release from the mitochondria (De Marco et al., 2006). It was also suggested that wild-type optineurin may have a role in neuroprotection in the eye and the optic nerve head (Rezaie et al., 2002). However, E3–14.7K has been shown to inhibit TNF- α -induced apoptosis and optineurin can reverse the E3–14.7K protective effect in human 293 cells (Li et al., 1998). Overexpression of optineurin also did not protect against transforming growth factor- β (TGF β)-induced apoptosis in β B1-crystallin-OPTN and β B1-crystallin-TGF β 1 double transgenic mice (Kroeber et al., 2006). Cell loss rather than protection was in addition observed upon overexpression of wild type and E50K optineurin in neuronal cells (Koga et al., 2010; Nagabhushana et al., 2010; Park et al., 2006). The cell loss was at least in part due to increased apoptosis, as evidenced by active caspase 3/7 staining (Koga et al., 2010). Apoptosis was moreover seen in RGCs of E50K transgenic mice (Chi et al., 2010). These different results from various investigations could be related to cell types/systems used, the level of optineurin expression, and/or the time points studied. Careful systematic

investigations are needed to resolve the issue whether, or under what conditions, optineurin is neuroprotective or neurotoxic.

5.2. Maintenance of Golgi organization

The Golgi apparatus is a highly dynamic cellular organelle composed of cisternal stacks. It transports, processes, and sorts macromolecules such as proteins synthesized in the rough endoplasmic reticulum for cell secretion (exocytosis) or use within the cell (Farquhar and Palade, 1998; Marsh and Howell, 2002). The Golgi stacks are usually arranged as an interconnected network in the region around the centrosome due to a microtubule-dependent mechanism. The organization of Golgi complex is maintained by at least four systems: microtubules and microtubule-associated proteins, the actin-associated cytoskeleton, the Golgi matrix proteins, and proteins that ensure the targeting and fusion of transport vesicles to the correct compartment, such as GTP-binding proteins and SNAREs (soluble NSF [*N*-ethylmaleimide-sensitive fusion protein] attachment protein receptor) (Gonatas et al., 2006).

The dissociation of the Golgi apparatus or Golgi fragmentation is a physiological and pathological reaction. During mitosis, Golgi complex is disassembled in early prophase and is readily reassembled in telophase (Robbins and Gonatas, 1964). The Golgi apparatus is also found fragmented or dispersed in a variety of neurodegenerative diseases, such as Alzheimer's disease (Baloyannis et al., 2009), Parkinson's disease (Fujita et al., 2006), ALS (Fujita et al., 2008; Mourelatos et al., 1994; Stieber et al., 2000), corticobasal degeneration (Sakurai et al., 2000), spinocerebellar ataxia type 2, and Creutzfeldt–Jakob disease (Gonatas et al., 2006; Sakurai et al., 2000). The cytoplasmic aggregation of mutant protein is the common feature in neurodegenerative diseases. These mutant protein aggregations may directly or indirectly interact with any of one or more proteins involved in the maintenance of the structure of the Golgi apparatus and disrupt its structure and function (Gonatas et al., 2006).

Optineurin is reported to play a role in the maintenance of Golgi organization (Park et al., 2006; Sahlender et al., 2005). When optineurin was depleted from cells via RNA interference, the Golgi became fragmented. The ribbon structure of interconnected stacks of membrane cisternae was broken up and the disconnected Golgi stacks were dispersed throughout the cytoplasm (Sahlender et al., 2005). On the other hand, when optineurin was overexpressed in human TM, RPE, and RGC5 cells, the Golgi complex also was disconnected or fragmented (Park et al., 2006; Ying et al., 2010). As stated earlier, when cells were transfected to overexpress optineurin–GFP fusion protein, bright granular or punctuate structures termed foci were observed in perinuclear regions of all cell types. They are dynamic, moving around in both short and long ranges (Ying et al., 2010). Subsequent experimentation using nocodazole proved that the formation of optineurin foci is microtubule dependent (Park et al., 2006; Ying et al., 2010).

The mechanism as to how optineurin is involved in maintaining the Golgi integrity is unknown. One hypothesis forwarded by Sahlender et al. (2005) is that optineurin might coordinate the actin cytoskeleton and microtubule-based motor activities for Golgi maintenance through interactions with its binding partners. Myosin VI, an actin-based motor protein, might link via optineurin to Rab8 which is involved in sorting molecules in the exocytic pathway at the trans-Golgi network (TGN) and in membrane fusion at the plasma membrane. Optineurin also binds with Htt which has been shown to bind with dynein as well as HAPI, a protein that interacts with dynactin p150 subunit and kinesin. The motor protein complex formed by these proteins is engaged in both anterograde and retrograde transport process (Caviston and Holzbaur, 2009; Rong et al., 2007; Sahlender et al., 2005). In the case of optineurin deletion or overexpression, the interaction between optineurin and its binding partners (such as myosin VI, Rab8, and Htt) may be altered, and the balance

between the motor proteins is disturbed to induce fragmentation of the Golgi complex (Fig. 5.4). The disturbance in balance may also be the basis why a reduction in the size of the Golgi complex was seen in Snell's waltzer mice. It was reasoned that the absence of myosin VI in those mice might cause pulling of the Golgi membrane by the dynein motor complex toward the microtubule organization center, resulting in a smaller, more compact Golgi apparatus (Sahlender et al., 2005; Warner et al., 2003).

Mutations in optineurin may also interfere with the optineurin interaction with its binding partners. When cells overexpressed a mutant that displays enhanced binding to Rab8 (Nagabhushana et al., 2010; Ying et al., 2010), such as E50K optineurin, the Golgi fragmentation was even more severe than the wild type (Park et al., 2006).

Again, much work remains to be done to unravel the mechanism(s). It does appear nevertheless that optineurin may need to be present in the cells in an optimal level such that all protein interactions are properly maintained. Disturbances of the various interactions, resulting from knockdown or mutation, or when optineurin is in excess, might have adverse consequences.

5.3. Vesicle trafficking

Vesicle trafficking plays a central role in the formation and maintenance of different intracellular compartments as well as in the communications between cells and their environment (Vassilieva and Nusrat, 2008). Major vesicle trafficking pathways consist of endocytosis and exocytosis. Protein trafficking by the endocytic pathway is an essential cellular mechanism critical for many functions including cell signaling, nutrient acquisition, and maintenance of the plasma membrane (Perret et al., 2005).

Optineurin-binding partners myosin VI, Htt, Rab8, and transferrin receptor are all involved in vesicular trafficking (del Toro et al., 2009; Hattula and Peranen, 2000; Park et al., 2010; Sahlender et al., 2005). The transferrin uptake, an indicator of the best characterized, clathrin-dependent, receptor-mediated endocytosis process, was examined in cells after transfection to overexpress wild-type optineurin. It was observed that the transferrin uptake was significantly decreased in optineurin-expressing cells (Park et al., 2010). The reduction was evident from the initial 2- and 5-min time points. Cotransfection with transferrin receptor, but not Rab8 or myosin VI, construct, rescued the optineurin inhibitory effect. The ectopic expression of optineurin caused transferrin receptor to colocalize with the optineurin foci (Nagabhushana et al., 2010; Park et al., 2010). Surface biotinylation experiments further showed that the surface level of transferrin receptor was lowered. It appears that the transferrin receptor molecules, via interactions with optineurin, are recruited and sequestered by the foci formed near the perinuclear area. This arrest may consequently lead to diminish availability of surface transferrin receptor and impeded transferrin uptake.

The E50K mutation, compared to the wild type, had an enhanced binding to transferrin receptor and yielded a more pronounced impairment in the transferrin uptake (Park et al., 2010). This mutation, generating much striking effects compared to the wild-type optineurin, is apparently a gain-of-toxicity mutation. The defective trafficking (Nagabhushana et al., 2010; Park et al., 2006), fragmentation of the Golgi complex (Park et al., 2010), and induction of apoptosis (Koga et al., 2010) are speculated to be the underlying bases why E50K mutation may render the patients predisposed to the glaucoma pathology.

A point mutation Leu¹⁵⁷ → Ala (L157A) in the leucine zipper region with which foci formation is minimal and interaction with transferrin receptor is unaltered has no inhibitory effect on the transferrin uptake (Park et al., 2010). Another point mutation D474N in the UBD domain similarly displays few foci and reduced interaction with transferrin receptor.

Transferrin molecules were internalized but were not trafficked to the perinuclear region (Nagabhushana et al., 2010). These findings imply that both the leucine zipper and UBD domains of optineurin may be required for the formation of foci as well as the interaction with transferrin receptor.

Using siRNA techniques, downregulation of optineurin was found to reduce significantly the transport of vesicular stomatitis virus G-protein to the cell surface and alter the morphology of Golgi complex in normal rat kidney and HeLa cells (Sahlender et al., 2005). Knocking down optineurin in RGC5 cells, as was seen with the overexpression, also compromised the transferrin uptake (Park et al., 2010).

There have been reports in the literature of various genes (Bache et al., 2003; Guilherme et al., 2004; Magadan et al., 2006; Morino et al., 2004; Park et al., 2009; Strick and Elferink, 2005) displaying identical or similar phenotypes under both overexpression and depletion conditions. For example, inhibition of transferrin internalization was seen to result from both overexpression and siRNA-mediated silencing of EHD2 (EH domain protein 2) (Guilherme et al., 2004). Genes Rab22a (Magadan et al., 2006), REP15 (Rab15 effector protein) (Strick and Elferink, 2005), and Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) (Bache et al., 2003; Morino et al., 2004) have also been noted to generate the same phenotype upon overexpression and knockdown: inhibited transferrin recycling, inhibited transferrin receptor recycling, and impaired degradation of epidermal growth factor, respectively. In the budding yeast, overexpression and deletion of Bud2 both resulted in a significant increase in the rate of actomyosin ring contraction (Park et al., 2009). In this last example, Bud2 overexpression was thought to act as a dominant negative. In the others, it was speculated that overexpression may lead to formation of unbalanced protein complexes, trapping proteins associated with endocytosis steps and inhibiting in turn their normal functions. This appears to be also the likely scenario involved in optineurin overexpression. Evidence is provided that the inhibition of transferrin uptake by overexpressed optineurin is mediated largely through the augmented optineurin–transferrin receptor interaction (Park et al., 2010). The precise mechanism involved in the depletion situation, however, still remains to be defined.

5.4. Regulation of NF- κ B signaling

NF- κ B is a transcription factor that controls a number of essential cellular functions including immune responses, cell proliferation, and antiapoptosis. NF- κ B is retained in an inactive form in the cytoplasm. Upon activation, the inhibitory proteins of the I κ B family are degraded following phosphorylation by the serine protein kinase IKK complex. NF- κ B is then dissociated from the inhibitors and translocated to the nucleus to trigger transcription of target genes.

The TNF- α -stimulated NF- κ B-dependent gene transcription is markedly enhanced when optineurin is downregulated (Zhu et al., 2009), indicating that optineurin functions normally as a negative regulator of NF- κ B (Sudhakar et al., 2009; Zhu et al., 2009). The mechanism by which optineurin regulates NF- κ B is, however, not completely defined. It may be related to NEMO, the catalytic unit known as IKK γ of the IKK complex, since optineurin shares a high amino acid homology with NEMO (Schwamborn et al., 2000). Optineurin has been shown to compete with NEMO to bind with ubiquitinated RIP which is necessary for efficient activation NF- κ B induced by TNF- α (Zhu et al., 2007). Once bound to polyUb RIP, optineurin may bind with another binding partner, deubiquitinase CYLD, to deubiquitinate polyUb RIP and thereby block the downstream reaction in the NF- κ B pathway, resulting in apoptosis (Nagabhushana et al., 2011). There appears to be a negative feedback loop; the optineurin gene expression is elevated by treatment of TNF- α via the NF- κ B pathway, and the optineurin induced in turn inhibits the NF- κ B activation,

suggesting a physiological role for optineurin in dampening the TNF- α signaling (Sudhakar et al., 2009; Zhu et al., 2007).

TBK1, an optineurin binding protein, can be recruited to the TNF receptor complex in response to TNF- α (Kuai et al., 2004) and activate NF- κ B -directed transcription (Bonnard et al., 2000). Whether or how optineurin is involved in the TBK1-related process is currently unknown. E50K optineurin has been reported to enhance the binding of optineurin to TBK1. Such an enhancement may lead to aberrant NF- κ B regulation and contribute as another factor in the development of glaucoma.

5.5. Antiviral signaling

Viral infection has been documented to stimulate innate immune response to produce various proinflammatory cytokines (such as type I interferons: IFN α/β) and chemokines to elicit an effective antiviral defense. Loss of IFN β signaling leads to severe immunodeficiency toward viral infection (Muller et al., 1994). Optineurin suppresses cytokine responses to viral infections. When optineurin was overexpressed in the cells followed by infection with RNA virus Sendai virus (SeV), the cells failed to express IFN β . Depletion of optineurin with siRNA by contrast promoted virus-induced IFN β production and decreased RNA virus replication (Mankouri et al., 2010). Immunoprecipitation and immunofluorescence studies identified optineurin in a protein complex containing the antiviral protein kinase TBK1 and ubiquitin ligase TRAF3. Mutagenesis studies (D474N mutation) showed that binding of ubiquitin was essential for both correct subcellular localization and negative regulation of IFN β by optineurin (Mankouri et al., 2010).

In another study, optineurin was noted to have antiviral activity through the viral TAX oncoprotein which is known to activate the NF- κ B pathway. When cells were infected with HTLV-1, optineurin was demonstrated to interact cooperatively with the TAX1 binding protein TAXBP1 to increase the ubiquitination of TAX1 along with TAX1-dependent NF- κ B activation and antiviral activity (Journo et al., 2009).

5.6. Antibacteria signaling

Optineurin is recently recognized as an autophagy receptor, functioning in innate immunity against cytosolic bacteria by linking the TBK1 signaling pathway to autophagic elimination of cytosolic pathogens (Wild et al., 2011). Optineurin directly binds with autophagy modifiers LC3/ GABARAP proteins and ubiquitin chains (Wild et al., 2011). Both the binding of optineurin to LC3 and selective autophagy of ubiquitin-coated cytosolic *S. enterica* are promoted after phosphorylation of optineurin by TBK1 at Ser-177 (Wild et al., 2011).

It appears that most *Salmonella* upon infection reside in *Salmonella*-containing vacuoles (SCVs) in the cells. A fraction of *Salmonella* may escape from the SCVs to the host cell cytosol where they hyperproliferate and serve as a reservoir for dissemination (Knodler et al., 2010). As a cellular defense mechanism, cytosolic *Salmonella* is rapidly coated with ubiquitin (Perrin et al., 2004). Optineurin is then recruited to the ubiquitinated cytosolic *Salmonella*. Following subsequent phosphorylation by TBK1, the LC3-optineurin binding is increased, and the phosphorylated optineurin can in turn act as a molecular trigger to promote autophagic clearance of cytosolic bacteria (Wild et al., 2011).

5.7. Induction of autophagy

Autophagy is another major route besides the ubiquitin–proteasome system for protein and organelle clearance in eukaryotic cells (Glickman and Ciechanover, 2002; Kirkin et al., 2009; McCray and Taylor, 2008). It is an evolutionally conserved mechanism responsible

for bulk degradation of long-lived proteins and cytoplasmic recycling of organelles during development, tissue homeostasis, and environmental stress such as starvation or amino acid depletion (Meijer and Codogno, 2009; Mizushima, 2007). There are three types of autophagy: macroautophagy, chaperone-mediated autophagy, and microautophagy (Todde et al., 2009). Among them, macro-autophagy (the most common form, often referred to as autophagy) is the one mediated by the organelle termed autophagosome. Upon an induction signal, macroautophagy (autophagy) starts when a flat membrane cistern wraps around a portion of cytosol and/or organelles, forming a closed double-membrane bound vacuole that contains cytoplasm. This vacuole, called autophagosome (Bao et al., 2010; Eskelinen, 2005; Mizushima et al., 2010), undergoes stepwise maturation processes that include fusing events with lysosomes to form autolysosomes (Dunn, 1990). The contents of autolysosomes are finally degraded by acidic lysosomal hydrolases and the degraded products are transported back to the cytoplasm.

Optineurin has been shown to bind with autophagic marker LC3 and is an autophagy receptor (Wild et al., 2011). In neuronal cells that overexpress wild type and E50K optineurin, the level of proteasome regulatory $\beta 5$ subunit (indicative of proteasome activity) is reduced and the level of LC3 is increased compared with mock transfected or nontransfected controls. Autophagosome formation was also detected by electron microscopy. The number of foci formed in optineurin overexpressing cells was found increased upon treatment of an autophagic inhibitor, 3-methyladenine, but decreased by treatment of autophagic inducer, rapamycin. These results indicate that the ubiquitin–proteasome function is compromised when optineurin is upregulated or mutated, while autophagy is induced and comes into play to degrade the overexpressed or mutated optineurin (Shen et al., 2010). In consistence with the conjecture, the turnover of overexpressed wild type or E50K optineurin is dramatically slowed down compared to the endogenous optineurin (Ying et al., 2010).

6. Concluding Remarks and Open Questions

Optineurin has attracted ample attention from investigators in a wide variety of fields due, in part, to its association with diseases such as glaucoma and ALS. Impressive advances have been made in the past few years regarding optineurin, affording a better understanding of the protein characteristics, binding partners, as well as its possible involvement in the Golgi maintenance, protein trafficking, and signal transduction in the cells. There are, however, still questions to be addressed. For instance, it is far from fully understood how mutations identified might affect cellular functions of optineurin and lead to disease phenotypes. Golgi fragmentation has been shown to be one of the causes of neural death in neurodegeneration (Fan et al., 2008). The mechanism why optineurin silencing or overexpression would trigger Golgi defect is unknown. Is Ca^{2+} homeostasis a player? Is the microtubule system involved?

Optineurin has many binding partners and evidently has multiple functions. Are there other novel interacting partners and yet-discovered optineurin functions? How are the various functions regulated? Protein aggregation is a common phenomenon in the neurodegenerative diseases such as Lewy bodies (α -synuclein) in Parkinson's disease, neuritic plaques (β -amyloid), and neurofibrillary tangles (hyperphosphorylated tau) in Alzheimer's disease, as well as Htt aggregates in Huntington's disease (Lee et al., 2011; Ross and Poirier, 2004; Selkoe, 2003). The endogenous optineurin can interact with itself to form hexamers and can also bind with partner proteins to form supermolecular complexes (Ying et al., 2010). Is optineurin an aggregation-prone protein? Will it form amyloid fibers? What are the conditions that drive optineurin to aggregation? Is the aggregate cytotoxic? What are the consequences? Finally, what is the role of autophagy in optineurin clearance and pathology?

Transgenic and optineurin knockout mice may be developed as powerful tools for further exploration of optineurin functions. Modern, emerging techniques such as proteomics and molecular biology manipulations will also greatly facilitate studies to resolve open questions and provide insights into the mechanisms directly involved in the disease development. Such information will be of critical importance in designs of target therapies and have high translational impact in treatment and prevention of optineurin-related diseases.

Acknowledgments

The authors thank Ms. Lisa Birmingham for assistance in illustrations and preparations of Figs. 5.3 and 5.4. The work was supported by Grant EY018828 and Core Grant EY001792 from the National Eye Institute, National Institutes of Health, Bethesda, Maryland.

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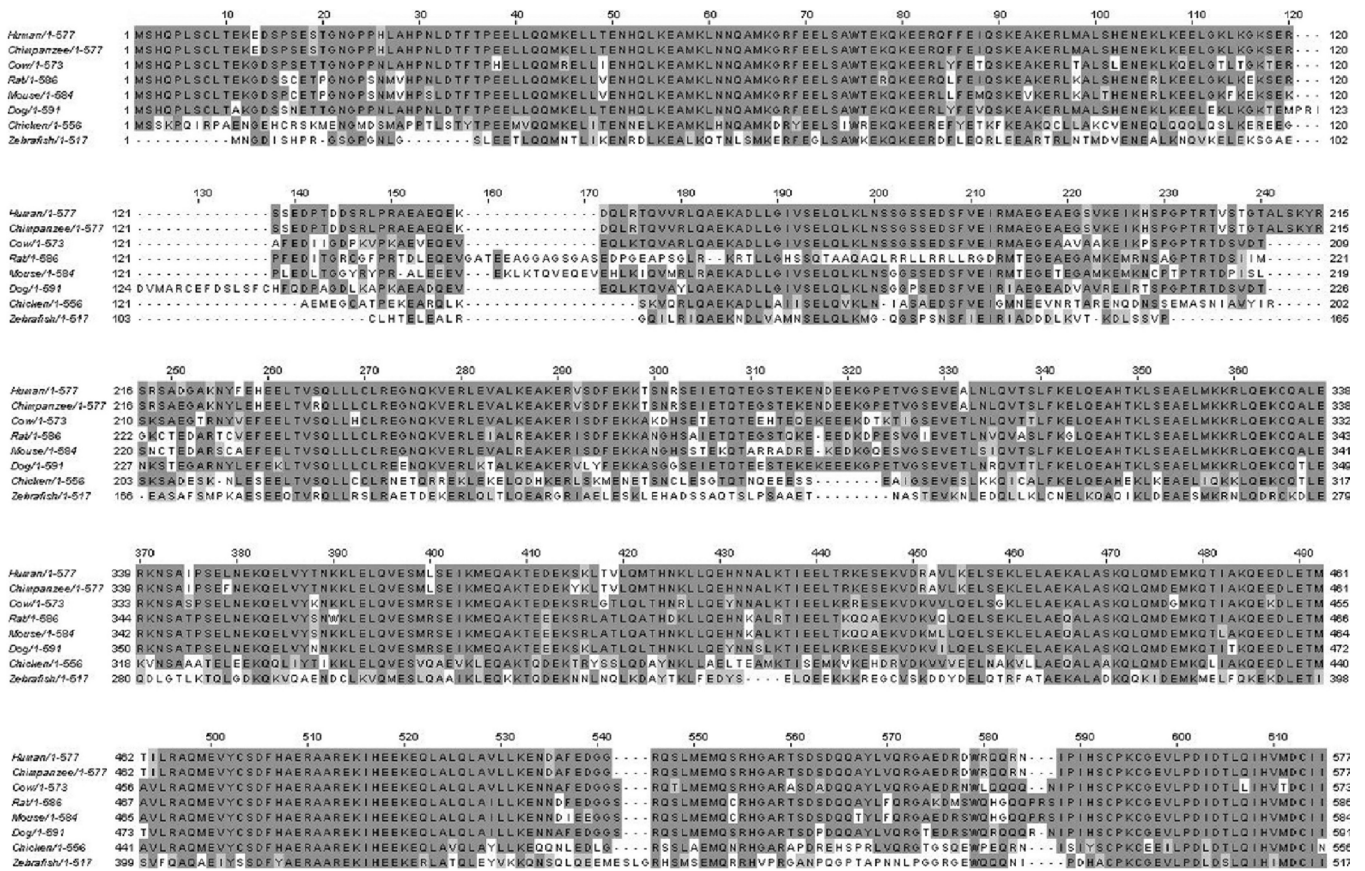


Figure 5.1. Sequence alignment of optineurin protein from human, chimpanzee, cow, dog, rat, mouse, chicken, and zebrafish. Multiple sequence alignment is generated by ClustalW2 (<http://www.ebi.ac.uk/msa/clustaw2/>).

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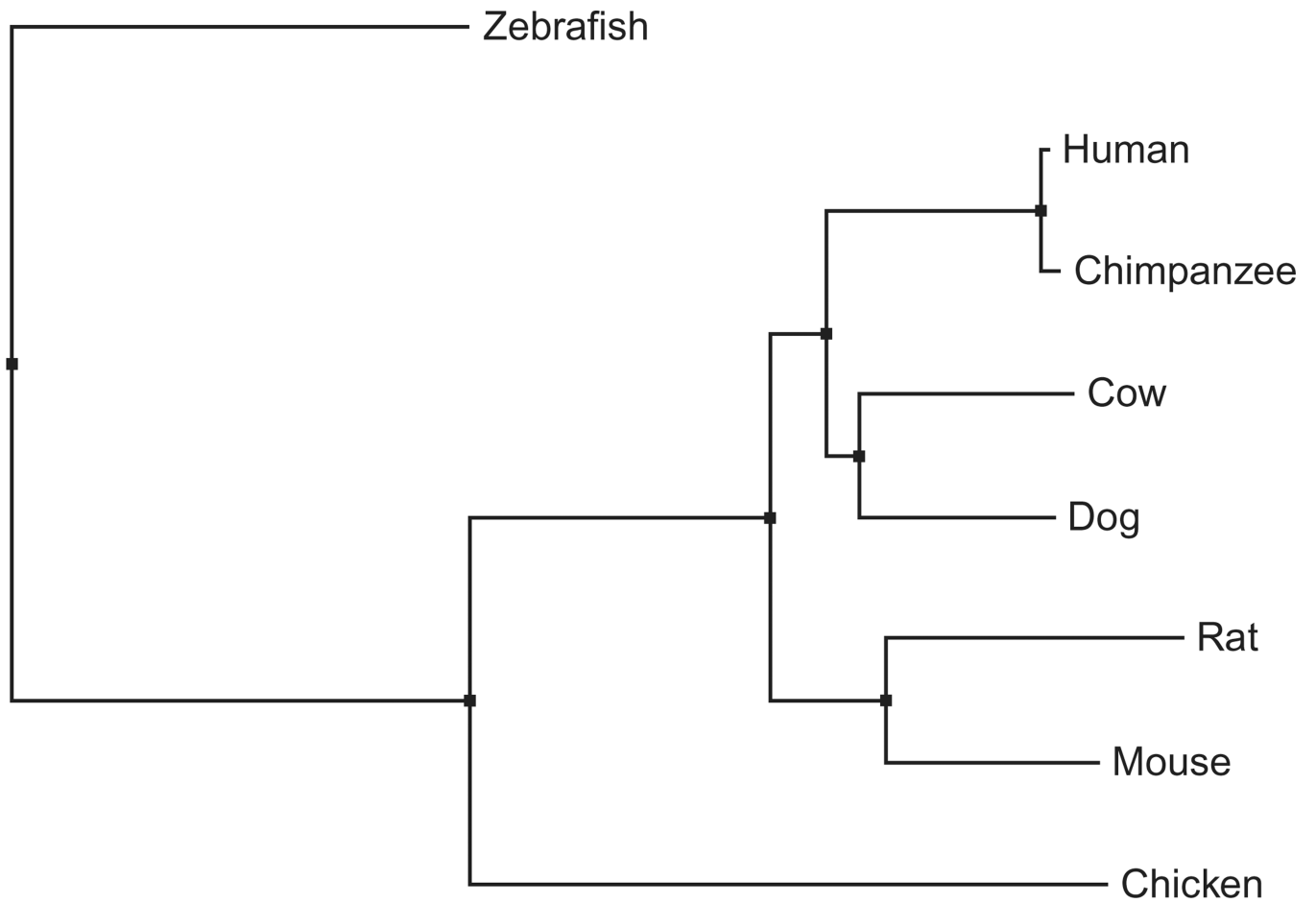


Figure 5.2. Phylogenetic tree to show the distance of optineurin protein between different species. Phylogenetic tree is generated by ClustalW2 (<http://www.ebi.ac.uk/msa/clustaw2/>).

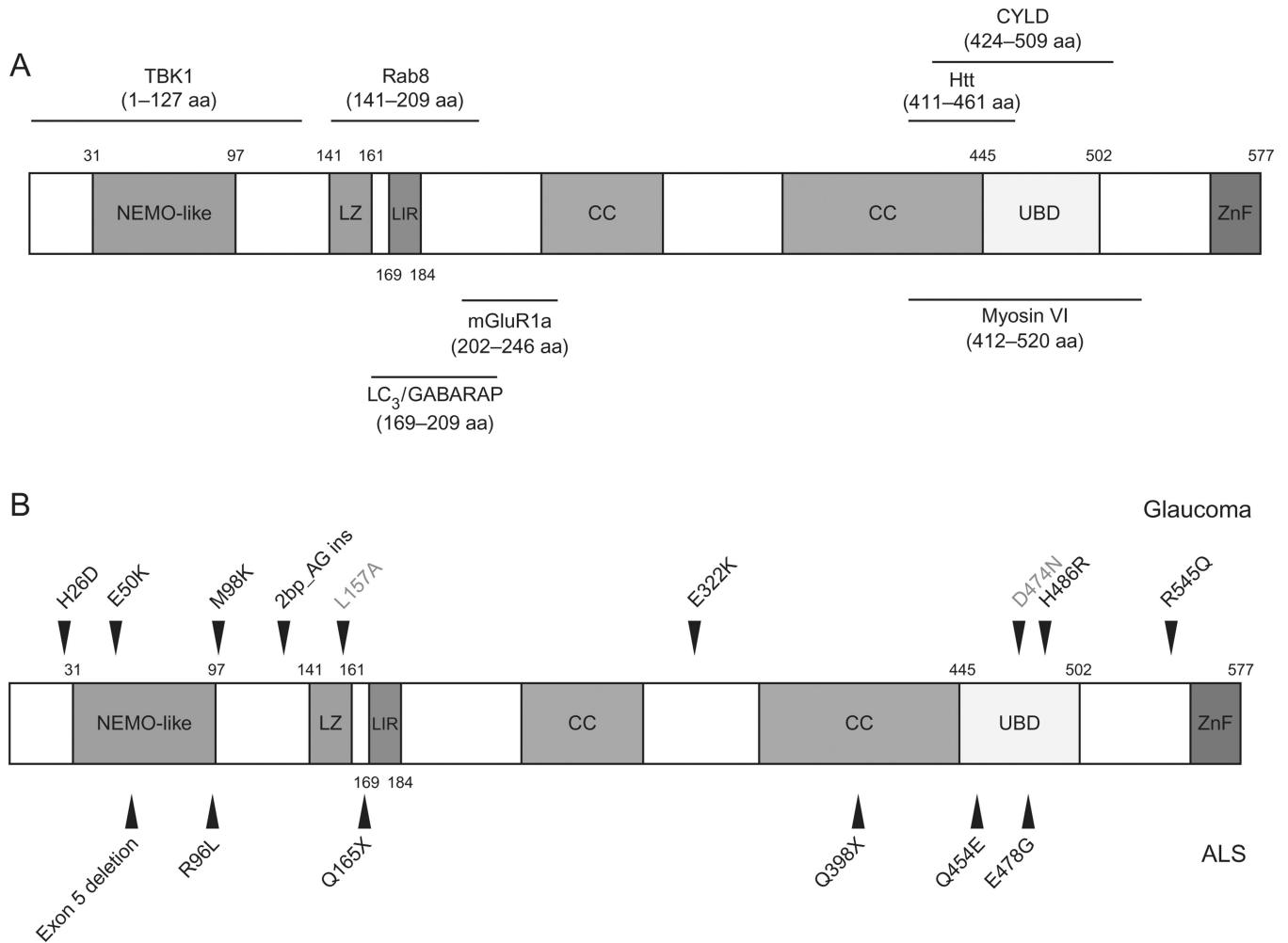


Figure 5.3.

(A) Schematic representation of human optineurin protein domains and the binding sites of optineurin-interacting proteins. CC, coiled-coil; LZ, leucine zipper domain; LIR, LC3 interacting motif; UBD, ubiquitin-binding domain; ZnF, zinc finger; aa, amino acid. (B) Optineurin mutations associated with glaucoma and ALS. Note that L157A is not disease associated but rather is a mutation used in the study to affect the leucine zipper domain. D474N also is not a disease associated mutation. It was used in studies to abolish ubiquitin binding. (For color version of this figure, the reader is referred to the Web version of this chapter.)

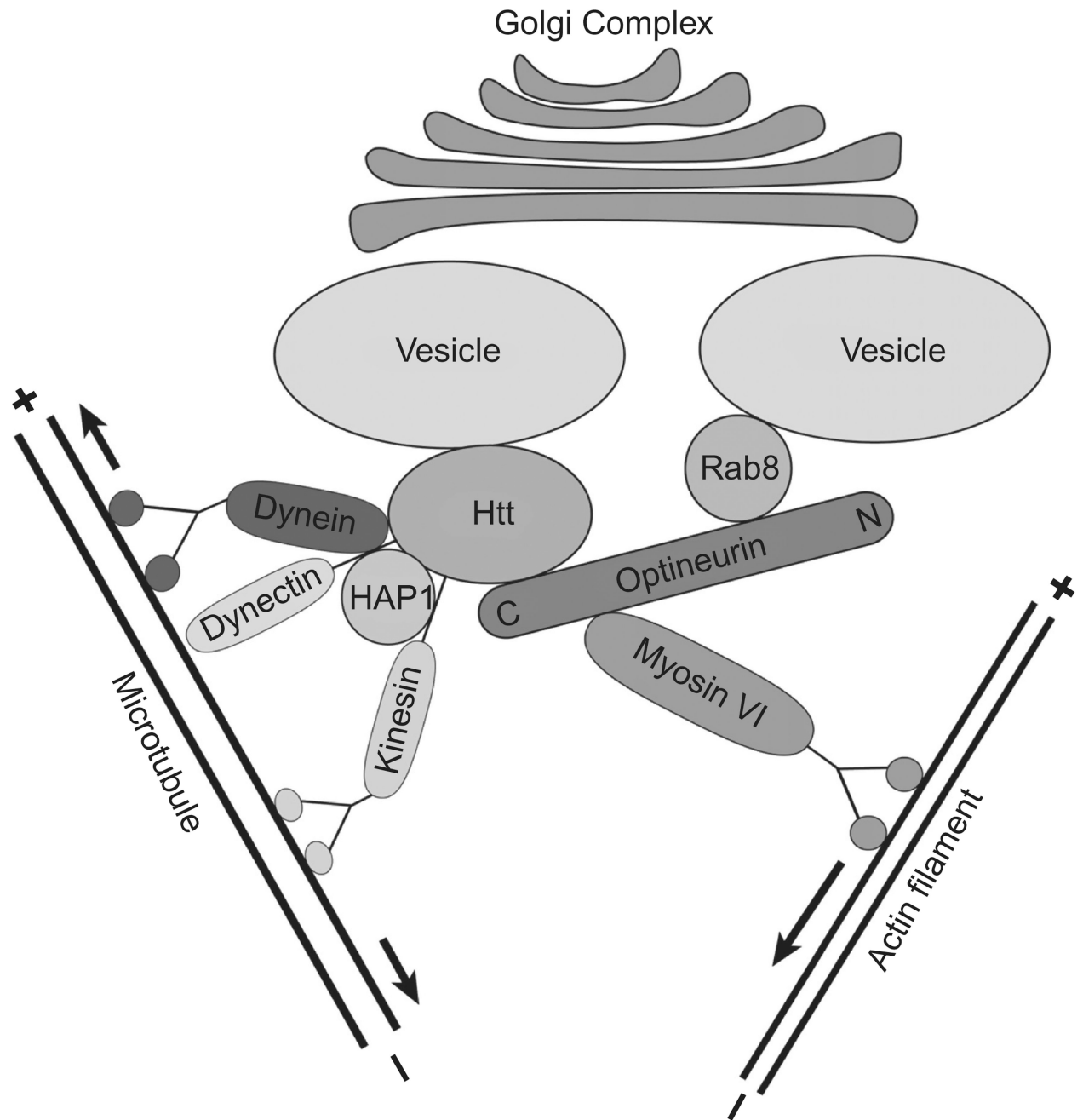


Figure 5.4.

Schematic model illustrating how optineurin may coordinate actin cytoskeleton and microtubule system for maintenance of the Golgi complex by interacting with various binding partners (modified from Sahlender et al., 2005 with updates). The C-terminal of optineurin binds with Htt which has been shown to bind with HAP1. HAP1 interacts with the plus (+)-end-directed microtubule motor protein dynein as well as dynein activator dynactin. The minus (-)-end-directed microtubule motor kinesin interacts with Htt and HAP1. Optineurin links Rab8 with myosin VI which is an actin-based motor protein. In scenarios of optineurin deletion or overexpression, the interaction between optineurin and its binding partners (such as myosin VI, Rab8, and Htt) may be altered and the balance between

the motor proteins may be disturbed to induce fragmentation of the Golgi complex. (For color version of this figure, the reader is referred to the Web version of this chapter.)